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EFFECTS OF SOME HEAVY METALS ON CHLOROPHYLL PIGMENT AND ASCORBIC ACID CONTENT IN INDIAN MUSTARD PLANT (*Brassic Juncea*) Leaves.

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ABSTRACT

Remediation of soil pollution is one of the many current environmental challenges. Plants can take out heavy metals from polluted soil and translocate them to their above plant tissues by the process of phyto extraction. Biochemical parameters in the leaves can be used as indicators of pollution for early diagnosis of stress and physiological damage. The present study was carried out to evaluate the effect of Cadmium (cd) and Lead (pb) on the chlorophyll and ascorbic acid content of *Brassica juncea* leaves. In this study, the pot experiment was performed in green house. *B. juncea* plants were planted in artificially Cd and Pb contaminated soils in different concentration of 10 $mgkg^{-1}$Cd($CdCl_2$), 50 $mgkg^{-1}$Pb ($PbCl_2$) and $mgkg^{-1}$Cd ($Cdcl_2$)+ 50 mgkg-^{1} ($PbCl_2$) along with ethylene diamenetetra acetate (EDTA) 1gkg^{-1}. *B. juncea plant* on exposure to Cd and Pb contaminated soil was showed the chlorophyll contents of the leaves were significantly decreased while the ascorbic acid content increase at the stage of flowering.

KEYWORDS: *Brassica juncea*, Lead, Cadmium, Chlorophyll, Ascorbic acid.

INTRODUCTION

Soil contains many heavy metals as a natural resource but along these excess of heavy metals come from anthropological activity. During last decades the heavy metal concentration such as Pb and Cd are increases in soil due to industrial activities. Cadmium is considered as one of the most dangerous metals because of its discharge, mobility and the small concentration necessary to show its effect on human health.[1] This increase in concentration of both essential and non-essential heavy metals in the soil can led to toxicity symptoms and growth inhibition in most pants.[2] In plants antioxidant system provides crucial protection against oxidative damage.[3] Lead and mercury was reported to cause an increase in ascorbic acid and α-tocopherol levels into Oryza sativa cultivars.[4] The present study reveals that the heavy metals Cd and Pb have effect on chlorophyll content and ascorbic acid in plants. Heavy metals interfere with chlorophyll content and ascorbic acid either by direct inhibition of enzymatic steps or by causing deficiency of essential components.[5]

MATERIALS AND METHODS

The soil was sampled in a depth of 0.15 cm from farming field area of Ambedkar Nagar near judicial Academy, Bhopal, M.P., dried indoors until it could be crumbled to pass through a 4 mm sieve for pots experiment and a 2 mm sieve for analyses of physicochemical properties. Soil samples were analyzed for, total organic carbon, available potassium or K_20, nitrogen or N and phosphorus or P_2O_5, concentration of Cadmium (Cd) and Lead (Pb)[5,6], concentration of Copper (Cu), concentration of Manganese (Mn), concentration of Nickel (Ni) and total bacterial count.[7] Soil was transferred to plastic pots (16 cm diameter and 20 cm depth) with drainage hole of 8 mm diameter, covered with a concave metal plate. Each soil sample was treated with 10 mg kg^{-1}Cd ($CdCl_2$). 50 mg kg^{-1}Pb ($PbCl_2$) and 10 mg kg^{-1}Cd ($CdCl_2$)+50 mg kg^{-1} Pb ($PbCl_2$) along with ethylene diaminetetra acetate (EDTA) 1g kg^{-1}. The soil contamination was performed by adding a specific amount of heavy metals; they were dissolved in deionized water into each pot (2 kg soil/pot) and then saturated, air dried at room temperature and thoroughly mixed. The wetting-drying mixing process was repeated to ensure soil equilibrium for one month period under natural light at a minimum temperature of 10-11^{0}C and maximum of 25-30^{0}C and a relative humidity of about 30-40%.

All experiments were performed with plants grown from the same seed batch. 5 seeds of *B.juncea, variety* Br 40 (obtained from Agriculture College, Sehore, M.P.), were sown in each pot with 5 replicates for each treatment. The experimental apparatus was housed under plastic shade that permitted good airflow and sunlight penetration but excluded incident rainfall. All experiments were conducted in the greenhouse under natural light. Air temperature ranged from 15 to 27^{0}C, the natural variation of the greenhouse. 10 seed were sown in each pot; after the first pair of true leaves appeared seedling was thinned to 5 plants per pot. All the treatment done in 5 replicate. After plant sowing, each pot was fertilized with N, P and K using urea (120 mg N kg^{-1}), Calcium phosphate (100 mg P kg-1), and Potassium sulphate (50mg N kg^{-1})as a basal fertilizing. For ascorbic acid and chlorophyll estimation the plant leaves were collected at three different growth stages i.e., 25 days (pre-flowering), 45 days (flowering) and 60 days (post flowering) for analysis.

Estimation of Chlorophyll Content

To study the phototoxicity, chlorophyll content of Mustard plant was studies, following the method of Arnon and Withom.[8] Pigments will be extracted from 100 mg fresh leaves in 80% acetone and the absorption at 665, 663, 649 and 626 mm were read in a spectrophotometer using the absorption coefficient and the amount of Chlorophyll was calculated. The amount of chlorophyll a and b present in the leaves extract was expressed in the terms of mg chlorophyll per gram leaves.

Estimation of Ascorbic Acid Content

Ascorbic Acid of fresh leaves of mustard plant (*Brassica juncea*) was estimated by volumetric method of Sadashivam.[9] Ascorbic acid reduces the 2, 6 dichlorophenol indophenols dye (DCPIP) to a colorless leuco-base. The ascorbic acid gets oxidized to dehydroascorbic acid, though the dye is blue colored compound, the end point is the appearance of a pink color. The dye is pink colored in acidic medium.

Statistical Analysis

SPSS software was applied to find the mean and standard error (S.E.) of the data. Replicate were 5 (n=5). To find the significance value, T-test was performed at 5% level of significance.

RESULT AND DISCUSSION

Heavy metals toxicity of Chlorophyll of *B. juncea* leaves

According to the Table: 1, the level of chlorophyll \`a\` and chlorophyll \`b\` in control plants were found to be maximum at the flowering stage i.e. 45 days in *B. juncea*. At flowering stage the chlorophyll \`a\` and \`b\` were decreased by 33% and 16% respectively on the concentration of 10 Cd. The chlorophyll \`a\` and \`b\` were decreased by 30% and 10% respectively on the concentration of 50 Pb at flowering stage. Pb proved to be less toxic as compared to Cd. The Chl \`a\` and Chl \`b\` were decreased to 37% and 18% respectively on the concentration of 10 Cd+50 Pb at flowering stage. Chl \`a\` get more affected by heavy metals as compared to Chl \`b\` (figure 1 &2). Chlorophyll content is often measured in the plants, in order to assess the impact of environmental stress. Visual symptoms of plant illness can be observed due to change in chlorophyll content and it also the cause of decrease chlorophyll in several different plant species under the impact of heavy metals.[10, 11] Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes for chlorophyll biosynthesis.

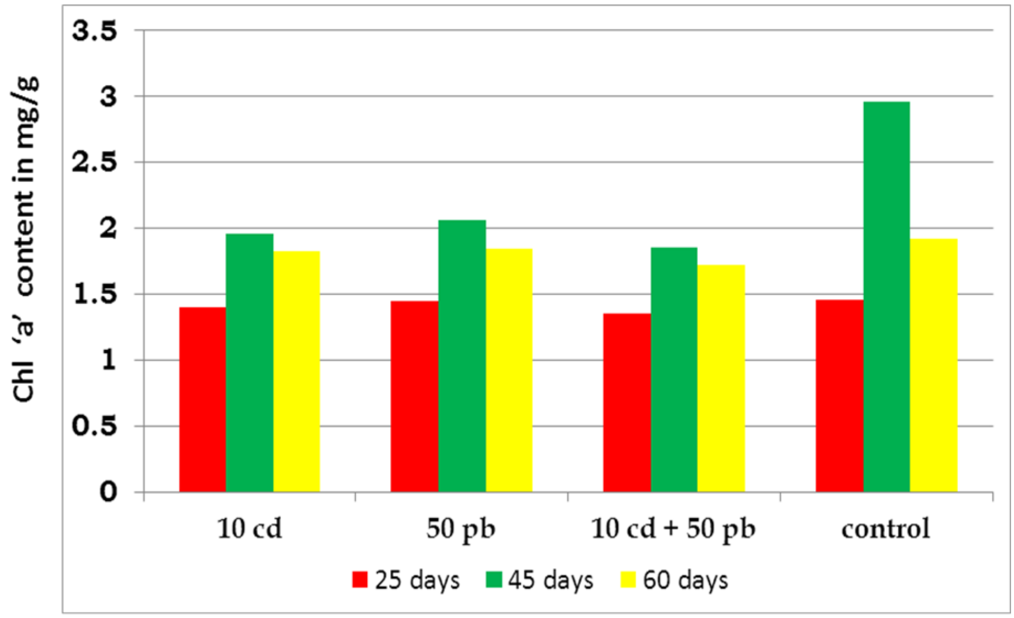
Table 1: Chlorophyll Estimation in *B.juncea* leaves growing on contaminated soil with Cd and Pb accompanied with EDTA at different developmental stages.

Concentrations Of heavy metals		Chlorophyll a (mg $^{g-1}$)			Chlorphyll b (mg g^{-1})	
25 days	45 days	60 days	25 days	45 days	60 days	
10 Cd	1.40±0.007	1.96±0.015	1.82±0.011	0.66±0.014	0.71± 0.007	0.64± 0.011
50Pb	1.45±0.016	2.06±0.011	1.84±0.010	0.70±0.008	0.75±0.008	0.69±0.007
10Cd+50Pb	1.35±0.013	1.85±0.015	1.72±0.011	1.72±0.011	0.59±0.01	0.62±0.011
Control	1.46±0.015	2.96±0.05	1.92±0.03	0.71±0.01	0.84±0.011	0.81±0.014

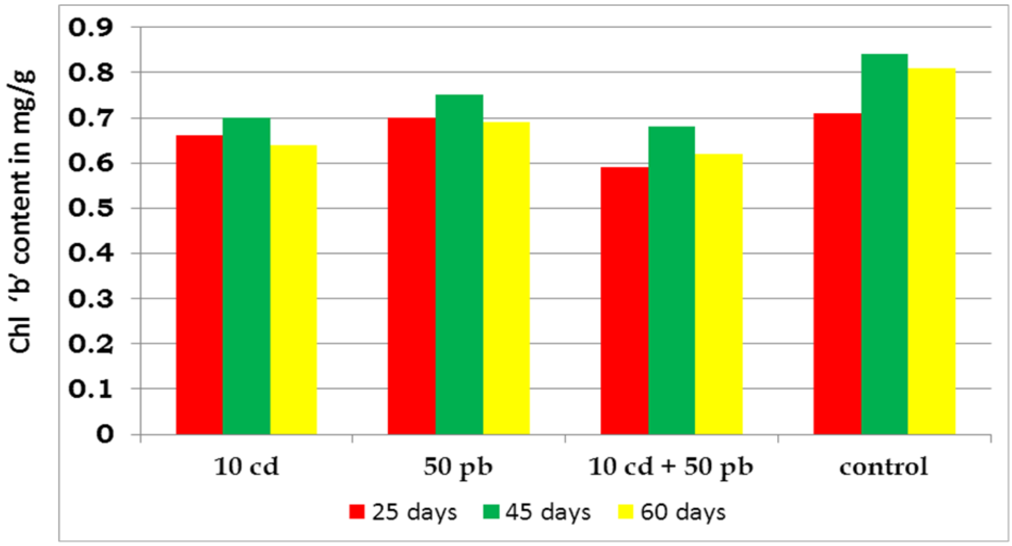
Mean±SE for all 5 individuals are significant at P>0.05

Cd: Cadmium Pb: Lead

Cholorophyll 'a' Estimation in *B.juncea* leaves with different concentrations of Cd and Pb at different developmental stages.



Cholorophyll ‘b’ Estimation in *B. juncea* leaves with different concentrations of Cd and Pb at different developmental stages



Cadmium was reported to affect chlorophyll biosynthesis and inhibit protochlorophyll reeducates and aminoevulinic acid (ALA) synthesis.[12] Total chlorophyll content in primary leaves decreased from 18.3% to 22.5% at 0.05, 0.06 and 0.08 mm concentrations of cadmium.[13] Our result supported with finding of Ahmed[14], who found that treatment of plant with lead reduced the chlorophyll contents.

Heavy Metals toxicity on Ascorbic Acid of *B. juncea* leaves

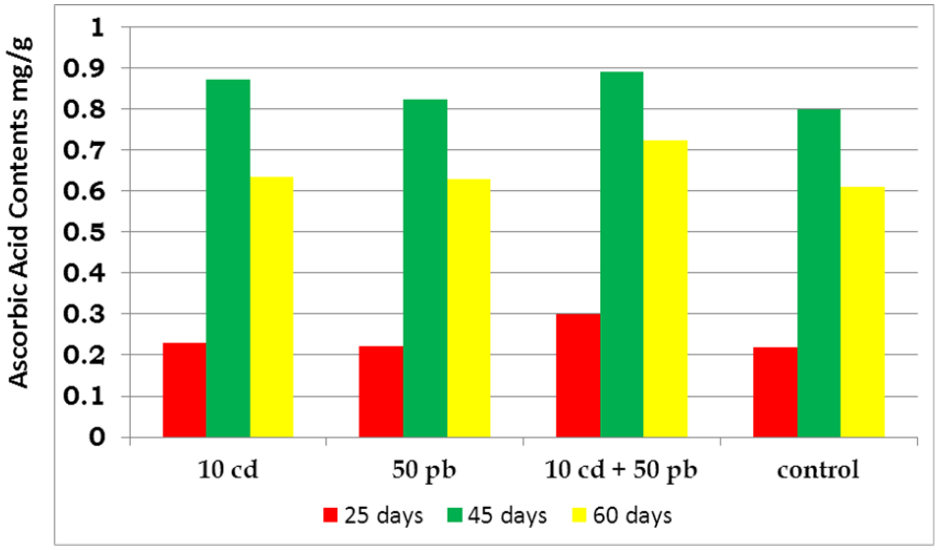
10 cd, 50 Pb caused increase in ascorbic acid by 4% and 3% respectively, while combination of 10cd+50Pb caused 18% increase in ascorbic acid at 60 days (post flowering stage). The increase in ascorbic acid was found to be at 45days (flowering stage) 9%, 3% and 11% at 10 Cd, 50Pb and combination of 10Cd +50Pb respectively. (Table: 2). The increase in ascorbic acid content at flowering stage was highest due to metal accumulation at flowering stage was more than the accumulation at pre-flowering stage (Figure:3)

Table 2: Ascorbic Acid Estimation of *B.juncea* leaves growing on contaminated soil with Cd and Pb accompanied with EDTA at different developmental stages.

Concentrations of Heavy metals	Ascorbic Acid contents (mg $^{g-1}$)		
	25 days	45 days	60 days.
10 Cd	0.229±0.016	0.872±0.033	0.635±0.019
50Pb	0.221±0.012	0.825±0.006	0.630±0.014
10Cd+50Pb	0.300±0.007	0.892±0.010	0.725±0.009
Control	0.218±0.006	0.800±0.005	0.610±0.003

Mean± A.E. for all 5 individual are significant at P>0.05

Ascorbic Acid Estimation in *B. juncea* leaves with different concentrations of Cd and Pb at different developmental stages.



In the study of Noctor and Foyer, the ascorbic acid content was increased to some extent because ascorbic acid acts as the antioxidant of pant, which protects the plant against adverse condition. Heavy metal stress to the plant may results in increase in antioxidant level. The antioxidant system comprises numerous enzymes and compounds of low molecular weight.[15]

Ascorbic acid of natural antioxidant in plants plays an important role in tolerance mechanism of the plant.[16] Lead and mercury was reported to cause an increase in ascorbic acid in Oryza Sativa.[17] Ascorbic acid increased by 12.7%, 13.2% and 14.2% on 1.5, 2.0 and 2.5 mg/gm lead concentration.[13] The result of above research supported our research study. Free radical generation is one of the initial responses of pants to stress. Generation of free radicals and reactive oxygen species is stimulated in the presence of metals[18], and this can seriously disrupt normal metabolism through oxidative damage to cellular components. To overcome this problem plants have developed a complex antioxidant system. Antioxidants like systeine, proline, ascorbic acid and non-protienthiol (Sulthydryl) play an important role in detoxification of toxic metal ions. Ascorbic acid is a ubiquitous soluble antioxidant in photosynthetic organisms, and the most important reducing substrate for H_2O_2 detoxification.

CONCLUSION

The conclusion of our study is that the exposure of *B. juncea* plant to Cadmium and Lead and Cadmium Lead combination spiked soil showed that chlorophyll contents of the leaves was significantly decreased at flowering stage. While in response to heavy metals stress, the antioxidant activity i.e. ascorbic acid content increased during the growth period as compared to the control leaves. The increase in ascorbic acid due to Cadmium toxicity was more than Lead toxicity. Our research work is also revealed that the Cadmium at 10mg Kg^{-1} showed the approximately same toxicity level as the Lead showed on 50mg kg^{-1}. While the combination of Cadmium and Lead together creates more toxicity to the plant than the toxicity creates by individual metals. This concluded that Cadmium was more toxic than Lead at lower concentration. *B. Juncea* extracts Cadmium more than Lead.

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